on both double- and single-stranded (ds and ss) DNA in presence of ATP, or a non-hydrolysable analog such as ATPγS. We here present studies of RecA-filaments, formed on either ds- or ssDNA, in a nanofunnel. The funnel geometry used allows probing the extension of a single filament at different channel dimensions, which in turn enables determination of the persistence length. The value we obtain for RecA on dsDNA, 1.15 ± 0.30 μm, agrees well with the literature. It is not straight-forward to obtain ssDNA that is long enough to allow studies in nanochannels. However, using rolling circle amplification (RCA) we were able to create ssDNA that is tens of thousands of bases. Using this ssDNA we estimate the persistence length to 1.44 ± 0.68 μm, slightly larger than what has been obtained for RecA-ssDNA filaments with conventional techniques.

Importantly, our experiments are performed in solution without the need to attach the DNA or protein to any "handles". This in turn means that the approach is directly applicable to most DNA-protein complexes and potentially also DNA-protein complexes extracted from cells.

2169-Plat

Dynamics of NAP1-Assisted Nucleosome Assembly Imaged with High-Speed Atomic Force Microscopy

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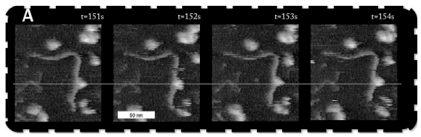
Nucleosome assembly is a vital part of chromatin maintenance for all eukaryotic cells. The histone chaperone NAP1 is one of the proteins involved in this process, guiding histones into the nucleus and assembling them into nucleosomes without use of ATP or other energy sources. It is known that NAP1 first brings H3H4 histone tetramers to the DNA to form a tetrasome, and in a second step H2a and H2B histones are added, but mechanistic insight into this process is still lacking. We use high-speed AFM to image NAP-1 assembled tetrasomes in vitro with spatial and temporal resolutions of the order of 1 nm and 1 s. We observe a rich palette of dynamical processes, among which are spontaneous tetrasome dissociation, cluster formation and a transient association between bare DNA and NAP1. One intriguing observation is the hopping of a tetrasome between two stable positions spaced only a few nanometers apart. We believe this motion can be ascribed to a re-orientation of the DNA around the histones, a phenomenon that we also observed in magnetic tweezer single molecule assays.



2170-Plat

Characterizing the Structure and Function of the N-Terminus of Schizosaccharomyces Pombe Cdc5, a Pre-Mrna Splicing Factor

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The spliceosome is a dynamic macromolecular machine composed of 5 small nuclear ribonucleoproteins (snRNPs), the Nineteen Complex (NTC), and other proteins that catalyze the removal of introns from pre-mRNA. The NTC is a highly conserved sub-complex of the spliceosome consisting of approximately fifteen proteins and is required for the transition of the spliceosome from an inactive to activated complex. However the mechanism of NTC function in this process is not understood. Schizosaccharomyces pombe Cdc5, a core subunit of the NTC, is an essential protein that contains two highly conserved N-terminal Myb repeats, a N-terminal non-canonical Myb-like repeat (MLR) as well as a less well conserved C-terminus. Both in vivo and in vitro studies have shown that the C-terminus of Cdc5 directly interacts with other NTC components; however, a specific function for the N-terminus of Cdc5 in pre-mRNA splicing has not been determined.

Our goal is to understand the specific role(s) of Cdc5 in NTC function and pre-mRNA splicing. Using a combination of yeast genetics, NMR secondary structure analysis, Chemical Shift (CS) Rosetta modeling, and an RNA binding assay we have shown the MLR is structurally distinct from the canonical Myb fold, that the N-terminus of Cdc5 preferentially binds double stranded RNA, and defined the importance the of the N-terminal Cdc5 Myb repeats and MLR in fission yeast.

2171-Plat

Condensation of DNA Mediated by the Bacterial Centromere Binding Protein Spo0J/ParB

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The condensation and dynamic re-organization of the chromosome is crucial to the cell cycle of all living organisms. In B. subtilis, the centromere binding protein Spo0J/ParB has recently been implicated in the recruitment of condensins at parS sequences close to the origin of replication [1, 2]. We have studied the binding of Spo0J to DNA molecules using magnetic tweezers. At reduced forces and high Spo0J concentrations, we observe a progressive condensation of the tethered DNA molecule. The condensation process is reversible upon increasing the force or removal of the protein, and shows a maximum stalling force of 3 pN at 8 μM Spo0J. Similar condensation curves were obtained for DNA substrates that either did or did not contain parS sequences, suggesting a non-specific binding mode of SpoOJ in agreement with complementary bulk studies. Control experiments using short competitor oligonucleotides confirmed that the condensation was Spo0J-mediated. Experiments using the freely orbiting tweezers technique and torsionally constrained DNA molecules show that the condensation process is accompanied by a preferred sense of rotation of the holding bead, but both clockwise and counter-clockwise rotations were found. We propose a model in which condensation is induced by interactions between neighbouring Spo0J proteins and these interactions are affected by the Brownian motion of the DNA tether.

References

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2172-Plat

Architectural Role of HMO1 in Bending, Bridging and Compacting DNA

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HMO1 proteins are abundant Saccharomyces cerevisiae (yeast) High Mobility Group Box (HMGB) proteins. HMGB proteins are nuclear proteins that are known to have an architectural function. HMO1 possesses two HMGB box domains. It has been reported that double box HMGB proteins induce strong bends upon binding to DNA. It is also believed that they play an essential role in reorganizing chromatin and therefore are likely to be involved in gene activation. To characterize DNA binding we combine single molecule stretching experiments and AFM imaging of HMO1 proteins bound to DNA. By stretching DNA bound to HMO1, we determine the dissociation constant and the cooperative parameter. Furthermore, we learn that HMO1 proteins form loops, and by pulling on these loops, we characterize the size of these loops and the average force to break a loop. Stretching experiments enable us to study the dynamics of loop formation and the time for reformation of these loops after they are pulled apart. AFM images are used to probe the conformations of individual HMO1-DNA complexes. The results show that at lower concentrations, HMO1 preferentially binds to the ends of the double helix and links separate DNA strands, forming bridges. At higher concentrations HMO1 induces formation of a complex network that reorganizes DNA. Taken together, these results suggest that both HMG boxes of HMO1 participate in multiple functions in vivo.

2173-Plat

Direct Visualization of DNA Dynamics During the Telomerase Catalytic Cycle Reveals the Function of a Conserved Telomerase Domain

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Telomerase is an enzyme that maintains the ends of eukaryotic chromosomes and is important to our understanding of both aging and cancer. The telomerase catalytic core contains both a conserved protein subunit known as telomerase reverse transcriptase (TERT), and a conserved RNA known as telomerase RNA (TER). Telomerase recognizes its DNA substrate by